# スペクトル解析ソフト SPANA© 簡易使用マニュアル

2005 年 8 月 8 日　　文責：谷間大輔

*注：SPANA© の著作権は、京都工芸繊維大学繊維学部高分子学科　黒田裕久教授にあります。そのことをお忘れなく…。このマニュアルは、黒田先生作成の英語版取扱説明書（5 ページ ～）の基本的な部分だけを日本語訳したものです。*

## 1．セットアップ

spana.zip を適当なフォルダ中に解凍して【setup】プログラムを実行する。
OS が Windows XP の場合はアイコンが画面上に出てこないので、ハード・ディスク上¥Program Files¥spana という新たに作られているフォルダ中にある【spana.exe】と言う実行ファイルのショート・カットをデスクトップ上に作って使用すると便利。

使用できるファイルは…

島津 UV ファイル (*.UVD)、島津 UV（ASCII フォーマット）ファイル (*.ASC)、日本分光 CD ファイル (*.JAC)、日本分光 UV ＆ 蛍光ファイル (*.TXT)

例えば…

## 2．日本分光 UV ファイルからの解析方法

1. インストールした際に作ったショートカットをダブルクリックし、SPANA© を開く。

2. 右側のカラムで、開きたいファイルのあるドライブとフォルダを指定し、File Type で*.TXT を選ぶ。

3. すべてのテキストファイルを選択し、【Load】→【Exit】→ 0～99Ch. までの“チャネル”が出現します。このチャネル上ですべての解析を行うので、必ず【Exit】しましょう。
【Channel】→【Load】で元に戻れます。

4. 解析を行ったすべての必要なチャネルを選択します。
☆例えば、30 点の滴定を行ったなら、それぞれのスペクトルデータが 0～29Ch. まで割り振られています。Shift キーを押しながら 0 と 29Ch. をシングルクリックすると全スペクトルが SPANA© の画面上に描き出される、という具合です。

6. スペクトルの作画範囲を必要な部分だけにします。
【View】→【Format】で、波長 (Wave) 及び吸光度 (Abs.) の最小値と最大値、そしてそれぞれのスケールを適度に決めます。
☆もし、データとして全く不必要で、その波長範囲は削りたい場合は…
【Channel】→【Item Edit】で、現在進行形の全チャネルの情報が出現するので、スタート(StartWave) あるいは終わりの波長 (EndWave) を全部入力する。【⇒ First Channel】に補正したデータを入れるチャネルを指定する。初めに 29Ch.までデータが入っているので、‘31 - ’という具合です。もちろん、重ね書きも OK です。重ね書きする場合は‘0 - ’ということですね。“ - (ハイフン)”はそのチャネル以降、それぞれのチャネルに順次データを割り振ることを意味しています。

7. ベースラインを揃えます。
【Spectrum】→【Base Line】→【Point Correction】で基準を揃えたい波長 (特に吸収が無い波長、例えば 800 nm) にカーソルを合わせ、シングルクリックする。【Ch.】‘All’⇒ 【Ch.】‘31 - ’と入力し、【OK】

8. 【View】→【Deactivate All/Cls】でいったんスペクトルをクリアし、ベースライン補正したデータ 31～61Ch. をクリックしてスペクトルを描き出し、次の補正に移る。

9. 希釈による濃度補正を行います（Lambert-Beer 則に則り推定の吸光度を計算してくれる）。
【Spectrum】→【Conc.Correction】で、【Original Volume (V)】にセルに入れたホスト溶液の初期量を入力します（単位：μl）。次に、それぞれの滴定点での滴下量を総量として入力し（単位：μl）、【⇒First Channel】に補正したデータを入れるチャネルを指定する。例えば、‘63 - ’と入力し、【OK】

10. 【View】→【Deactivate All/Cls】でいったんスペクトルをクリアし、濃度補正したデータ 63～93Ch. をクリックしてスペクトルを描き出し、次の補正に移る。

11. 最小二乗法で解析を行うために、差スペクトルをとります。
【Spectrum】→【－】→【Channel】で、【Ch.】‘All’－【Ch.】‘63’（ハイフンはつけない）（基準となる初期溶液のデータ）⇒【Ch.】‘0 - ’（差スペクトルをどこから収めるか）と入力し、【OK】

12. 【View】→【Deactivate All/Cls】でいったんスペクトルをクリアし、差をとったデータ 0～29Ch. をクリックしてスペクトルを描き出し、いよいよ最小二乗法を用いた解析を行えます。

と、その前に…
すべての補正を行ったデータをここで保存しましょう。次回からは、そのデータを用いて更なる解析を SPANA© で楽しめます。
【File】→【Save As…】で、“File Name”を入力します。デフォルトでは‘*.ana’（SPANA© で開ける拡張子）となっていますが、拡張子はそのままで適当にファイル名を決め、“Directory”で保存する場所を選択します。

13. 最小二乗法を用いた解析を行います。

①【Analysis】→【Least Square Analyses…】→【Point Data…】で、カーソルが出現するので解析を行いたい波長に合わせ、ダブルクリックします。すると、‘Result’として全スペクトルに対応する吸光度のテーブルが出ます。

☆SPANA では多変数解析も行えます。例えば、566, 368, 265 nm の 3 点で解析を行いたい場合、【Point Data…】で出てきたカーソルを 566, 368 nm ではシングルクリックし、最後の 265 nm でダブルクリックすると、先ほどと同様のテーブル（カラムは 3 つに増える）が出てくる、という具合です。

②テーブル上で、【Least Square Analyses】を選ぶと出てくる【Equilibrium Model】の項で“1 : 1 Complexation Model”または“1 : 2 Complexation Model”のうち、‘Known $Abs_A$’の‘Diluted[A]’にチェックマークを入れ、【Go】*!!!!!*

1 : 2 Complexation Model の場合…

③“Least Square Optimization”が現れるので、‘Experimantal Condition:’には、Conc.of A（ホスト溶液の濃度）、Initial Volume of A（セルに入れた初期量）、Conc.of B（ゲスト溶液の濃度）を入力する。

☆例えば、1.5 x $10^{-4}$M の場合・・・“**1.5 e -4**”と入力する。（**e** は自然対数の意味ではありません）

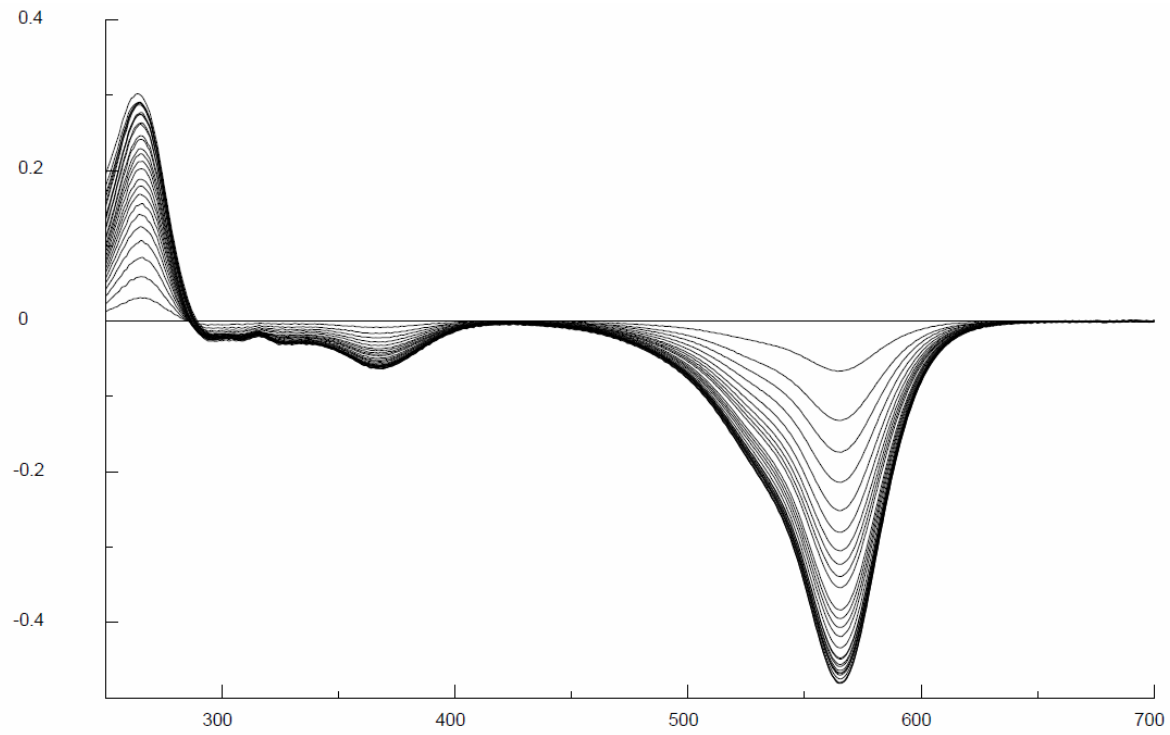
④‘Initial Guess of Parameters’には、予想される会合定数 $K_1$ 及び $K_2$、モル吸光係数 $\varepsilon$ を入力します。その値が的外れでなければ、“Calc.”をクリックすると、適当な値に収束してくれるはずです♪

収束せず、エラーメッセージが出る場合は、値を入れ直します。

⑤さらに、“Least Square Optimization”上で‘Plot’をクリックすると、“Graph Format”が現れ、縦軸と横軸に適当な値を入力すると、最小二乗法でのプロットを描き出してくれます。滴定がどれくらいの錯形成率のところを見ているのか（飽和しているか否か）を確認できます。

⑥“Plot”上で、【Graph】→【Print All】を選択すると、4 ページのように、補正したスペクトル、最小二乗法で解析して得た会合定数及び ε 、錯形成率のプロットを PDF 形式で印刷してくれます。

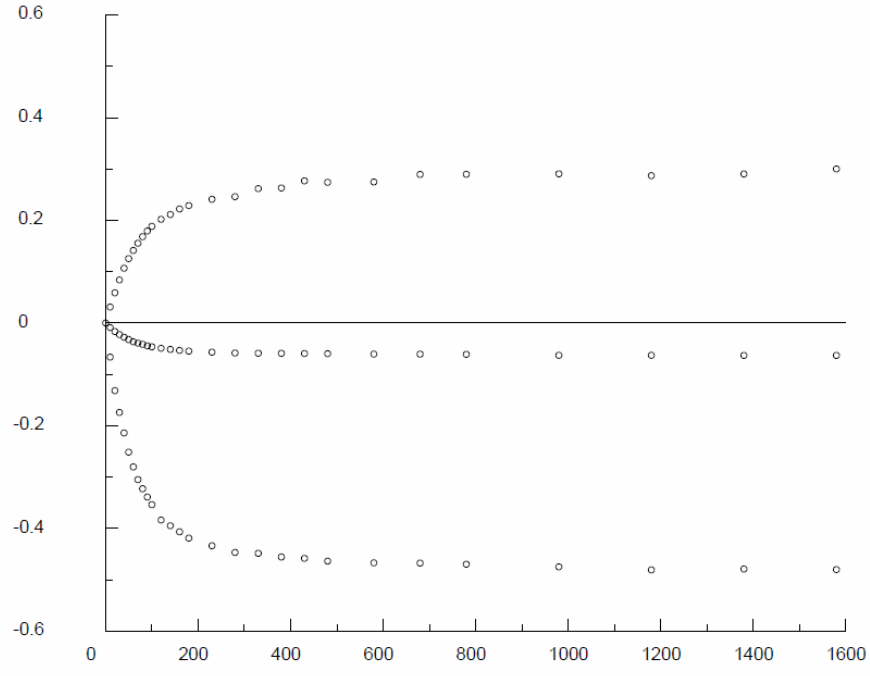
以上で一通りの操作は完了です。。。




Least Square Optimization
Data at 566 , 368 , 265 ,nm.
Optimized Parameters (Iteration: 1 , AIC:-627.4594)

K1 = 15244.82 (sd: 1344.529)
De1 = -5389.252 (sd: 214.4928)
De2 = -695.0817 (sd: 34.48552)
De3 = 2507.605 (sd: 135.2337)
K2 = 2835.223 (sd: 412.9589)
De*1 = -5928.281 (sd: 36.44315)
De*2 = -776.2584 (sd: 15.83876)
De*3 = 3806.412 (sd: 26.13815)

Residual Square Sum : 4.711986E-04



# SPANA v. 2.03

## Spectrum Analyses Program

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Yasuhisa Kuroda
Department of Polymer Science
Kyoto Institute of Technology
Matsugasaki, Sakyo, Kyoto 606-8585
JAPAN

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If you have any comments, questions or suggestions please do not hesitate to contact the author:

Yasuhisa Kuroda,
Department of Polymer Science
Kyoto Institute of Technology
Matsugasaki, Sakyo, Kyoto 606-8585
JAPAN
Tel.&Fax. +81-75-724-7830
Email: **ykuroda@kit.ac.jp**

**Introduction**

SPANA is a spectrum analyses program intended for manipulations and analyses of the electronic, emission, and related spectra. The program is written and compiled with Microsoft Visual Basic and runs on standard Microsoft Windows 95 or 98. The system equipped with 32 MB memory or higher is recommended for smooth operation.

The program reads spectrum files including Hewlett Packard (HP) 8453 UV spectra, Shimazu UV spectra , JASCO CD spectra and Hitachi Fluorescence spectra. SPANA has 100 channels (Ch.0 - Ch.99) for spectrum storage, which have the 180-1100 nm ranges with the 0.5 nm digital resolution.* All manipulations of the spectra are performed for the data loaded on these channels.
(* If the loaded spectra have different digital resolution, SPANA adds neccesary data generated by interpolation using adjacent four data points.)

**General Operation**

**Setup and Running SPANA**

The setup of SPANA starts from double clicking on the "setup" icon and following the program's instructions. After completion of the setup processes, the SPANA icon will be added in your program folder. To start SPANA under Microsoft Windows, double-click on the SPANA icon. When SPANA starts, the program displays a single main window consisting of a display area with a black background and a dialogue box for file loading operations at the right side of the window. The main window can be resized by usual Windows operations.

**File loading**

SPANA identifies spectrum files with their file extensions. SPANA currently supports the following file formats;

| | |
|---|---|
| Shimazu UV file : | *.UVD |
| Shimazu UV file (ASCII format) | *.ASC |
| Shimazu time course file : | *.3D |
| Hitachi fluorescence file : | *.FSD |
| HP UV file : | *.WAV |
| HP time course file : | *.TIM |
| JASCO CD file | *.JAC |
| JASCO UV & fluorescence text file | *.TXT |
| SPANA standard file : | *.ANA |
| SPANA high resolution file : | *.HRS |

The format of the file to be loaded is selected from the "File Type" list box. Selecting the drive and the directory which contains your spectrum files, the user can load necessary spectrum files appearing on the list box. Two method to load the spectra into the channels are available;

* double click on the file (the corresponding file name is highlighted) :

→ the single spectrum is loaded and displayed on the spectra display area.

* single click on several files (the corresponding file names are highlighted) and
click the “Load” command button :

→ all highlighted files are loaded and displayed on the spectra display area.

In order to select successive files, single click on the first file (the corresponding file name is highlighted) and single click again with shift key down on the second file shown below the first one (all file names between first and second ones are highlighted). Each loaded spectrum is stored automatically in the youngest empty channel, which is named with the file’s name.

To exit the file loading dialogue area, click the “Exit” command button and the channel list box will appear. To go back to the file loading dialogue area, select “**Channel-Load**” menu from the menu bar of the window.

**SPANA’s Operations**

All channels in the channel list box may have one of three possible states, “empty”, “activated” and “deactivated”. Only activated channels are highlighted and their spectra are displayed on the screen. The activated and deactivated states can be interchanged each other by single click on the channel. Activation and deactivation of all channels may be done by the menu “**View-Activate All**” and “**View-Deactivate all/Cls**” respectively. For activation of successive channels, the method similar to that for file loading are available, i.e., single click on the first channel (the corresponding channel is highlighted and activated) and single click again with shift key down on the second channel shown below the first one (all file names between first and second ones are highlighted and activated).

**Most of SPANA’s operations, modification, analyses and data read-out, are performed for these activated channels and ignore the deactivated or empty channels.**

All operations are menu-driven and the necessary information for each operation is inputted from the appropriate dialogue box given during the operations. The currently available menus are give below.

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| **Files** | **Channel** | **Spectrum** | **Analyses** |
|---|---|---|---|
| **New** | **Load** | **+*** | **Wave Separation** |
| **Save As** | **Remove** | **-*** | **Auto…** |
| **Print** | **Move** | ***** | **Manual** |
| **Exit** | **Copy** | **/*** | **Regression Analyses** |
| | **Exchange** | **Conc. Correct** | **Component Analyses** |
| | **Channel Edit** | **Shift** | **Least Square Analyses** |
| | **Item Edit** | **Base Line** | **Point Data** |
| | | **Point Correction** | **Area Data** |
| | | **Average Correction** | **Area Data in k/cm Unit** |
| | | **Linear Lines** | |
| | | **Curved Lines** | |
| | | **Gaussian** | |
| | | **Lorenzian** | |
| | | **Diffrential** | |
| | | **1st ~ 5th** | |

**Smooth**

| | |
|---|---|
| **View** | **Data Read** |
| **Activate All** | **Overlap Area** |
| **Deactivate All/Cls** | **Point Data** |
| **Zoom On** | **Area Data** |
| **Format** | **Area in k/cm Unit** |
| **View in k/cm Unit** | **About** |

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* These arithmetical operation menus have sub-menus, "**Constant**", "**Channel**" and "**Table**".

The menu "*****" has a further additional sub-menu, "**Wave Num. (v)**".

During the following operation, various types of dialogue boxes are shown. In each dialogue box, the "tab" key may be used for changing the "focus" of the cursor and the "Enter" key for click of "OK" command button.

**Function of Menu**

In the most of the channel modification processes, the over-write operations on the channels are allowed to yield the expected results.

**Files**

**New**

Clear all channels and show the file loading dialog area.

**Save As**

Save all data in channels. SPANA has two file formats, "*.ana" and "*.hrs", of which digital resolutions are 1 nm and 0.5 nm respectively. The names of the file, drive, directory are assigned in the shown dialog box.

**Print**

Print the spectra shown on the display. The paper direction (portrait or landscape) and the width of the wave and absorbance axis are assigned in the shown dialog box.

**Exit**

Exit SPANA.

**Channel**

**Load**

Change the dialog mode at the right side of the program window from the channel list box to the file loading box.

**Remove**

Remove the spectra in the activated channels and make the empty channels.

**Move**

Move the spectra of the activated channels to the successive channels of which start channel is assigned in the shown dialog box.

**Copy**

Copy the spectra of the activated channels to the successive channels of which start channel is assigned in the shown dialog box.

**Exchange**

Exchange the spectra of the activated channels with those of the successive channels of which start channel is assigned in the shown dialog box.

**Channel Edit**

Each channel of SPANA has the channel data for the “start” and “end wave length”, “condition” and “comment” as the additional independent information. Although these data are usually set automatically, the user can change these data of the activated channels manually in the shown dialog box. The channel data for the “condition” item are used as the independent variable for the “Least Square Calculation” (see below).

**Item Edi**t

Change the values of a single item in the channel data of the activated channels. The item which should be changed is selected by the radio buttons on the shown dialog box.

## Spectrum

**+ (plus)**

This and following three menus have three sub-menus, “**Constant**”, “**Channel**” and “**Table**”.

**Constant**

A constant value is added to each spectrum in the activated channel. The dialog box for this operation shows four input boxes, “Ch.Num.”, “value”, “Resulting Ch.Num.” and “Comment”. First, determine the target channels for the “Ch.Num.” box. The default value of Ch.Num is “All”. The key word “All” means the all activated channels are selected for calculation automatically. If the user wants to identify a single specific channel, input the channel number in this box. Second, the value to be added is input in the next “value” box. The channels for the resulting spectra are assigned in the third box. When the user use the “All” key word for the activated channels, the key word “-” (hyphen) in addition to the channel number is applicable for this input. By using hyphen such as “10-”, the necessary channels for the calculation results are automatically acquired at the successive channel 10, 11, 12, and etc. For example, when Ch. 5,7,9,10 are the active channels, the combination of following input values:

Ch. All + 0.5 → Ch.15-

results in

Ch.5 + 0.5 → Ch.15, Ch.7 + 0.5 → Ch.16, Ch.9 + 0.5 → Ch.17, Ch.10 + 0.5 → Ch.18. The channel comments for the resulting channel are also automatically generated by adding “+” to the original channel. In the above example, the channels comment of Ch.16 is “Ch.7+”. The user, of cource, also identify a single specific channel. If the user does not use the combination of the keyword “All” and the hyphen, necessary channel numbers and channel comments must be input manually.

**Channel**

A specific spectrum is added to each spectrum in the activated channel. The procedures are almost same with those of the “Constant” menu. The channel number which contains the spectrum to be added is input in the second box instead of the numerical value.

**Table**

Series of numerical values are successively added to spectra of activated channels. For example, for calculation, Ch.5 + a, Ch.7 + b, Ch.9 + c, Ch.10 + d, the values, a, b, c, and d, are input in the table shown in the dialog box. The modified spectra are set successively from the channel assigned for the result channel.

**- (minus)**

A constant value or spectrum is subtracted from each spectrum in the activated channel. The menu and the procedures are same with those for “+”.

*** (multiply)**

Each spectrum in the activated channel is multiplied by a constant value or spectrum.The menu and the procedures are same with those for “+”. This menu has another additional sub-menu ;

**Wave Num. (v)**

For the spectra f(ν) in active channels, calculate $f(\nu)\ \nu^n$ ,where ν is wave number in $kcm^{-1}$ unit. The value of n is given in the dialogue box. The negative n is acceptable. Combining this operation with the area calculation operation described below, the integration of the type of

$$\int f(\nu)\nu^n d\nu$$

is easily estimated.

**/(divide)**

Each spectrum in the activated channel is divided by a constant value or spectrum The menu and the procedures are same with those for “+”.

**Conc.Correct**

This menu is a special version of the “***-Table**” menu for titration data. Correct successive titration spectra by dilution factors;

corrected spectra = original spectra * (V + v) / V

where V is the original volume of the sample and v is the added volume of the titrant. The modified spectra are successively set from the channel assigned for the result channel. The vales of “v” are set as the “condition” parameter of the channel information (see **Channel-Channel Edit** menu).

**Shift**

The spectra in the activated channels are shifted by a given wave length (nm). The minimum unit is 0.5 nm.

**Base Line**

**Point Correction**

The spectra in the activated channels are corrected so that the absorbances at a single wave length position become zero. The position is defined by the “line cursor-single click” operation on the screen. The result channels are set by the same procedure with those for the arithmetical modifications.

**Average Correction**

The spectra in the activated channels are corrected so that the averaged absorbances within an assigned area become zero. The area is assigned by two line cursors (single click) on the screen. The result channels are set by the same procedure with those for the arithmetical modifications.

**Linear Lines**

Generate connected multi-lines. The points to generate the multi-lines are assigned by the cross cursor (single click) on the screen. After defining all necessary points, the “double-click” on the screen shows the dialogue box to assign the result channel which stores the generated multi-lines.

**Curved Line**

Following curve genaration is carried out based on the wave number unit such as “$cm^{-1}$“( not on the wave length unit such as “nm” ).

**Gaussian**

Generate a gaussian line defined with three points given by the user. These points are defined by single-click of the cross-cursor on the screen. After defining three points, the gaussian curve and a dialog box appear on the screen. On click the “OK” button, another dialogue box appears to indicate the result channel which stores the generated gaussian. If you click the “Cancel” button on the first dialog box, the gaussian will disappears and the operation for the definition of another gaussian starts again.

**Lorenzian**

Generate a Lorenzian line defined with three points given by the user. The procedures are same with those for “**Gaussian**”.

**Differential - 1st, 2nd, 3rd, 4th, 5th**

These differential menus generate the differential spectra in the activated channels. The result channels are set by the same procedure with those for the base line correction. The spectra's width of the “n” th differential spectra narrows by “n” nm at the both ends.

**Smooth**

Smooth the spectra in the activated channels. The degree of smoothing is regulated by the smoothing point parameter shown in the dialog box. The spectra's width of the “n” point smoothed spectra narrows by “(n ±1)/2” nm at the both ends.

## Analyses

**Wave Separation**

**Auto**

A spectrum is automatically separated by using Gaussian or Lorenzian functions. The spectrum area to be separated is assigned by two line cursors (single click) on the screen. This operation is applied only for a single spectrum which has the youngest channel number in the activated channels. The separated Gaussian or Lorenzian waves are stored in the successive channels which the user assigns in the dialog box.

**Manual**

The Gaussian or Lorenzian comoponents for wave separation are assigned manually. The operations for definition of the wave components are similar to those for **Base Line** - **Curved Line**. Assign several Gaussians

or Lorenzians by clicking the “Set” command button on the shown dialog box. The final wave component is defined by click of the “OK” button. The residual operations are same with those for the **Auto** mode.

**Regression Analyses**

(cf. Blackbum, J. A., *Analytical Chem.*, **1965**, *37*, 1000. Leggett, D. J., *ibid*., **1977**, *49*, 276
Minami, S., “Wave Data Analyses for Scientifc Measurement” (Japanese), CQ Press, Tokyo Japan **1986** )

Carry out the multiple regression analysis for the spectral components of the mixed spectra by using a set of standard spectra. The target spectra, the result channel, and the standard spectra (max. 10 spectra) used for analyses are assigned in the dialog box. After analysis, the ratios of each standard spectrum are shown in the Table form and the simulated spectrum composed by the resuts is set in the result channels as the “simulated spectra of Ch. x”. The single-click of the left button of the mouse on this channel shows the simulated spectrum. The single-click of the right button of the mouse on this channel shows the all spectra contained in the simulated spectrum. (The ratios of each standard spectrum may be also edited from the “**Channel-Channel Edit**” dialog box.)

**Component Analyses**

(cf. See above references.)

Estimate the number of independent eigenvalues (spectra) for a set of spectra. The resulting eigenvalues are shown in the table and the graph of log(eigenvalue) vs. number of eigenvalues.

**Least Square Analyses**

Carry out the least square analyses using equilibrium or kinetic models for the data sampled out from the spectra in the activated channels. The spectral data are used as the dependent variable and the values in the “condition” item of the channel as the independent variable. Following three sampling methods are available.

**Point Data**

The absorbance or intensity data of the spectra in the activated channels at a specific wave length assigned by the line cursor are sampled out. The user may assign up to 10 positions. The read-out data are shown in the table dialogue box. The data for the “condition” items may be changed directly in this table by numerical key-in procedures. The user may print, save, plot these data, and go to the least square calculation from this table dialogue box.

* The “Plot” command generates the new graph window plotting the data of the intensity or absorbance on the y-axis. The values in the “condition” item of the channels are used as the data for the x-axis (see **Channel-Channel Edit** menu). The graph is drawn according to the the format (x-width, y-width, and their scales) assigned in the dialogue box. The resulting graph can be printed out from the “**Print**” menu of this window.
* The “Least Sq.” command first shows the dialogue box where the model for least square calculation is selected. Following 8 equilibrium models and two kinetic models are currently available:

Equilibrium Model: Complexation between two species, A and B, where A is titrated with B.

1) 1:1 Complex formation model,
$A + B \leftrightarrow AB$, $K_1=[AB]/[A][B]$
where the initial concentration $[A]_{Total}$ is kept constant during the titration.
Theoretical relationship; $\Delta Abs = Abs_{obs} - \varepsilon_A[A]_{Total} = \Delta\varepsilon\,[AB]$, $(\Delta\varepsilon = \varepsilon_{AB} - \varepsilon_A)$
Used data set ; $\Delta Abs$ as dependent variable (y-axis) , $[B]_{Total}$ as independent variable (x-axis)
Optimized parameters ; $K_1$, $\Delta\varepsilon$

2) 1:1 Complex formation model,
$A + B \leftrightarrow AB$, $K_1=[AB]/[A][B]$
where the initial concentration $[A]_{Total}$ is diluted with the titrant solution during the titration.
Theoretical relationship; $\Delta Abs = \{(V + v)/V\}Abs_{obs} - \varepsilon_A[A]_{Total} = \{(V + v)/V\}\Delta\varepsilon\ [AB]$
where V is the initial volume of the solution of A, and v is the added volume of the titrant.
Used data set ; $\Delta Abs$ as dependent variable (y-axis) , v as independent variable (x-axis)
Optimized parameters ; $K_1$, $\Delta\varepsilon$

3) 1:1 Complex formation model,
$A + B \leftrightarrow AB$, $K_1=[AB]/[A][B]$
where the initial concentration $[A]_{Total}$ is kept constant during the titration but $\varepsilon_A[A]_{Total}$ can not be obtained experimentally.
Theoretical relationship; $Abs_{obs} = \varepsilon_A[A] + \varepsilon_{AB}\ [AB]$
Used data set ; Abs as dependent variable (y-axis) , $[B]_{Total}$ as independent variable (x-axis)
Optimized parameters ; $K_1$, $\varepsilon_A$ , $\varepsilon_{AB}$

4) 1:1 Complex formation model,
$A + B \leftrightarrow AB$, $K_1=[AB]/[A][B]$
where the initial concentration $[A]_{Total}$ is diluted with the titrant solution during the titration.and $\varepsilon_A[A]_{Total}$ can not be obtained experimentally.
Theoretical relationship; $\{(V + v)/V\}Abs_{obs} = \{(V + v)/V\}(\varepsilon_A[A] + \varepsilon_{AB}\ [AB])$
Used data set ; Abs as dependent variable (y-axis) , v as independent variable (x-axis)
Optimized parameters ; $K_1$, $\varepsilon_A$ , $\varepsilon_{AB}$

5) 1:2 Complex formation model,
$A + B \leftrightarrow AB$, $K_1=[AB]/[A][B]$ , $AB + B \leftrightarrow BAB$, $K_2=[BAB]/[AB][B]$
where the initial concentration $[A]_{Total}$ is kept constant during the titration.
Theoretical relationship; $\Delta Abs = Abs_{obs} - \varepsilon_A[A]_{Total} = \Delta\varepsilon_1\ [AB] + \Delta\varepsilon_2\ [BAB]$,
($\Delta\varepsilon_1 = \varepsilon_{AB} - \varepsilon_A$ , $\Delta\varepsilon_2 = \varepsilon_{BAB} - \varepsilon_A$)
Used data set ; $\Delta Abs$ as dependent variable (y-axis) , $[B]_{Total}$ as independent variable (x-axis)
Optimized parameters ; $K_1$, $K_2$, $\Delta\varepsilon_1$ , $\Delta\varepsilon_2$

6) 1:2 Complex formation model,
$A + B \leftrightarrow AB$, $K_1=[AB]/[A][B]$ , $AB + B \leftrightarrow BAB$, $K_2=[BAB]/[AB][B]$
where the initial concentration $[A]_{Total}$ is diluted with the titrant solution during the titration.
Theoretical relationship;
$\Delta Abs = \{(V + v)/V\}Abs_{obs} - \varepsilon_A[A]_{Total} = \{(V + v)/V\}(\ \Delta\varepsilon_1\ [AB] + \Delta\varepsilon_2\ [BAB])$
where V is the initial volume of the solution of A, and v is the added volume of the titrant.
Used data set ; $\Delta Abs$ as dependent variable (y-axis) , v as independent variable (x-axis)
Optimized parameters ; $K_1$, $K_2$, $\Delta\varepsilon_1$ , $\Delta\varepsilon_2$

7) 1:2 Complex formation model,
$A + B \leftrightarrow AB$, $K_1=[AB]/[A][B]$ , $AB + B \leftrightarrow BAB$, $K_2=[BAB]/[AB][B]$
where the initial concentration $[A]_{Total}$ is kept constant during the titration but $\varepsilon_A[A]_{Total}$ can not be obtained experimentally.
Theoretical relationship; $Abs_{obs} = \varepsilon_A[A] + \varepsilon_{AB}\ [AB] + \varepsilon_{BAB}[BAB]$
Used data set ; Abs as dependent variable (y-axis) , $[B]_{Total}$ as independent variable (x-axis)
Optimized parameters ; $K_1$, $K_2$, $\varepsilon_A$ , $\varepsilon_{AB}$ , $\varepsilon_{BAB}$

8) 1:1 Complex formation model,
$A + B \leftrightarrow AB$, $K_1=[AB]/[A][B]$ , $AB + B \leftrightarrow BAB$, $K_2=[BAB]/[AB][B]$
where the initial concentration $[A]_{Total}$ is diluted with the titrant solution during the titration.and $\varepsilon_A[A]_{Total}$ can not be obtained experimentally.
Theoretical relationship; $\{(V + v)/V\}Abs_{obs} = \{(V + v)/V\}(\ \varepsilon_A[A] + \varepsilon_{AB}\ [AB] + \varepsilon_{BAB}[BAB])$
Used data set ; Abs as dependent variable (y-axis) , v as independent variable (x-axis)

Optimized parameters ; $K_1$, $K_2$, $\varepsilon_A$ , $\varepsilon_{AB}$, $\varepsilon_{BAB}$

Kinetic Model;

1) 0 th Oder reaction

Theoretical relationship;$Abs = kt + Abs_0$

Used data set ; Abs as dependent variable (y-axis) , t (time) as independent variable (x-axis)

Optimized parameters ; k , $Abs_0$

2) 1st Order reaction

Theoretical relationship ; $Abs = Abs_0\exp(-kt)$ or $Abs = Abs_0(1-\exp(-kt))$

Used data set ; Abs as dependent variable (y-axis) , t (time) as independent variable (x-axis)

Optimized parameters ; k , $Abs_0$

* The "Go" command shows the dialogue for parameter setting for the least square calculation. The number of necessary parameters is determined from the user's sampling points and the calculation model. The Damping Gauss-Newton method is used for the non-linear least square calculation (L.S.). After setting the necessary values of the condition determinants such as the concentration of the sample, the user must input initial guesses for the optimized parameters. The user can change the damping factor (the default value is 1), if necessary. The "Calculate" command executes L.S. and shows the resulting values of the optimized parameters, their standard deviations, and the residual square sum. Then, the user may print out the results, plot the data and the resulting theoretical curve by the corresponding commands. The plot form shown here is similar to that described for the data plot procedure.

**Area Data**

The data for the area (in nm•absorbance or intensity unit) assigned with two cursors are used for L.S. as the independent variable. The procedures for L.S. are same with those described above.

**Area Data in k/cm Unit**

The data for the area (in $kcm^{-1}$•absorbance or intensity unit) assigned with two cursors are used for L.S. as the independent variable. The procedures for L.S. are same with those described above.

## View

**Activate All**

Activate all channels containing the data.

**Deactivate All/Cls**

Deactivate all channels and clear the screen.

**Zoom On**

Change the "Zoom" mode of the screen to "ON " (the menu checked) or "OFF" (the menu unchecked). In the zoom mode, the user can assign the zooming area by the click-drag operation on the screen. The "mouse button-up" operation shows the dialog box indicating the assigned area. The any other line cursor operations such as the "**Base Line**" correction cancels the "Zoom" mode, automatically.

**Format**

Change the format (wave length-width, absorbance or intensity-width, and their scales) of the spectra. This operation also cancels the "Zoom" mode, automatically.

**View in k/cm Unit**

The spectra are shown in the $kcm^{-1}$ unit (the menu checked). In this mode, the user can not use any cursor operations and change the spectral format. The spectra can be print out in this format. For exit this mode, select this menu again (the menu unchecked).

## Data Read

**Overlap Area**

Calculate the area overlapping between two spectra in $kcm^{-1}$•absorbance or intensity unit.

**Point Data**

Same with the corresponding menu in the "**Least Square Analyses**" menu, except that the option for L.S. is not available.

**Area Data**

Same with the corresponding menu in the "**Least Square Analyses**" menu, except that the option for L.S. is not available.

**Area in k/cm Unit**

Same with the corresponding menu in the "**Least Square Analyses**" menu, except that the option for L.S. is not available.

**About**

Show the "About" box.